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MATROCLINIC INHERITANCE IN MUTATION CROSSES OF OENOTHERA REYNOLDSII¹

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INTRODUCTION

This paper is concerned primarily with the peculiar type of inheritance exemplified among the mutations of *Oenothera Reynoldsii*, a species elsewhere described as showing the phenomenon of "mutation *en masse*," or mass mutation. It has been found that the mutations characteristic of mass mutation in this species, when crossed among one another, or with the parent form, give crosses which in general conform exactly to the type of the pistillate parent, quite regardless of which way the cross may have been made.

De Vries² has shown that in *Oenothera Lamarckiana*, the most thoroughly studied of the evening-primroses, the total number of mutations lies in the neighborhood of 2.2 percent. Certain mutations from *Oe. Lamarckiana* are themselves more mutable than their parent. Thus *Oe. lata* produces twice, and *Oe. scintillans* three times as many mutations as *Oe. Lamarckiana* itself. Before the discovery of mass mutation in *Oe. Reynoldsii* and *Oe. pratincola*, a form was considered highly mutable if its progeny contained as many as five or six percent of mutations. Aside from *Oe. Lamarckiana*, however, only one species, *Oe. biennis*, had been extensively grown for the detection of mutability,

¹ Prior to 1915 the work upon which this paper is based was carried on by the Office of Physiological and Fermentation Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and since then by the University of Michigan. Published by permission of the Secretary of Agriculture.

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² De Vries, *Gruppenweise Artbildung*, p. 329 *et seq.*

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and this species had been shown by Stomps³ and De Vries⁴ to be less mutable than *Oe. Lamarckiana*. In the recently discovered mass-mutating species the number of mutations may rise to almost 100 percent of the progenies.

The elementary species that have thus far shown mass mutability are both segregates from the collective species that passes in our floras as *Oenothera biennis*. True *Oe. biennis* seems to be found in America, but the records in regard to its occurrence have not yet been published. It is therefore not incorrect to state that the species (in the narrow sense) is definitely known only in Europe, where it occurs as an introduced weed. The name *Oe. biennis* has been applied correctly by De Vries and Stomps, but very loosely indeed by American geneticists, with the result that the literature is considerably confused. *Oe. Reynoldsii* and *Oe. pratincola* are two, among a number of segregates from the collective species of the floras, that have been described and named⁵ for the purpose of keeping a clear record of the genetical experiments that are being carried out with them. They are not recognized in current systematic works.

The first paper dealing with *Oenothera Reynoldsii*⁶ was written before any mutation crosses had been made. It was therefore only natural to suggest that the whole series of mutations to which it was giving rise were probably Mendelian recessives. Work on the closely related segregate *Oe. pratincola* shortly afterward disclosed the fact that the mutations characteristic of mass mutation were not Mendelian recessives, but showed matroclinic inheritance in crosses with their parent form.⁷ It has now been determined that the first suggestion in regard to the mutations of *Oe. Reynoldsii* was entirely erroneous, since they likewise show matroclinic inheritance. Although the special purpose of this paper is to present the data in regard to matroclinic inheritance, there is one other striking discovery which it is possible to announce at this time, namely, that in *Oe. Reynoldsii*, as

³ Stomps, Theo. J., Mutation bei *Oenothera biennis* L., Biol. Centralbl. 32: 521-535. 1912; Parallele Mutationen bei *Oenothera biennis* L., Ber. Deutsch. Bot. Ges. 32: 179-188. 1914.

⁴ De Vries, H., The Coefficient of Mutation in *Oenothera biennis* L., Bot. Gaz. 59: 169-196. 1915.

⁵ Bartlett, H. H., Twelve Elementary Species of *Onagra*, Cybele Columbiana 1: 37-56. 1915.

⁶ Bartlett, H. H., Mutation *en masse*, Amer. Nat., 49: 129-139. 1915.

⁷ Bartlett, H. H., Mass mutation in *Oenothera pratincola*, Bot. Gaz., 60: 425-456. 1915.

well as in *Oe. pratincola*, the occurrence of mass mutation is associated with a remarkable increase in seed sterility. This very significant fact is being made the subject of further study. The degree of seed sterility in mass-mutant *Oe. Reynoldsii* is much greater than in *Oe. pratincola*, and is so marked that otherwise indistinguishable individuals, the one stable, the other mass-mutant, can easily be distinguished by an examination of the seeds.

Without going into detailed repetition of data published in the former paper on *Oe. Reynoldsii*, it may be recalled that the wild form of the species, f. *typica*, has given rise to the derivatives mut. *semialta*, mut. *debilis*, and mut. *bilonga*. The f. *typica* is remarkable in that it exists in two morphologically identical phases, one of which is relatively stable, whereas the other is mass-mutant, giving rise to polymorphic progenies containing all of the mutations enumerated, as well as others which have not yet been carefully examined. Mut. *semialta* was so named because the plants of the early cultures, grown in Maryland, were about half as high as f. *typica*. The cultures of the season of 1916, grown in Michigan under other environmental conditions, did not show so great a disparity in height, but in other respects the forms were no less distinct than before. The shape of f. *typica* is depressed-conical, because of the long, widely spreading lower branches, whereas mut. *semialta* has relatively erect lower branches and is therefore somewhat cylindrical rather than conical in shape. Mut. *debilis* is a weak dwarf with much reduced foliage. Mut. *bilonga* was so named because its fruits are twice as long as those of mut. *semialta*, which it closely resembles in form and stature. In other respects, however, it will be shown that mut. *bilonga* more closely resembles mut. *debilis*, from which it springs, than mut. *semialta*.

All the mutations come true from seed, except that mut. *semialta* is capable of giving rise to mut. *debilis*, and that the latter may in turn give rise to mut. *bilonga*. Mut. *semialta* has once thrown a mutation which will be known as mut. *rigida*. It came entirely true in a large progeny grown in 1916, and will receive a larger share of attention in a future paper. A few other types have appeared in the cultures, but it has not been possible to obtain seeds from them.

SUMMARY OF THE CULTURES

Figure 1 is a chart giving the pedigree of all the cultures of *Oe. Reynoldsii* and its mutations that have thus far been grown from self-

pollinated seeds. Each progeny represented in the chart has a key number which serves to identify it with the detailed analysis of the same progeny in Table I. Several of the earlier progenies were not as

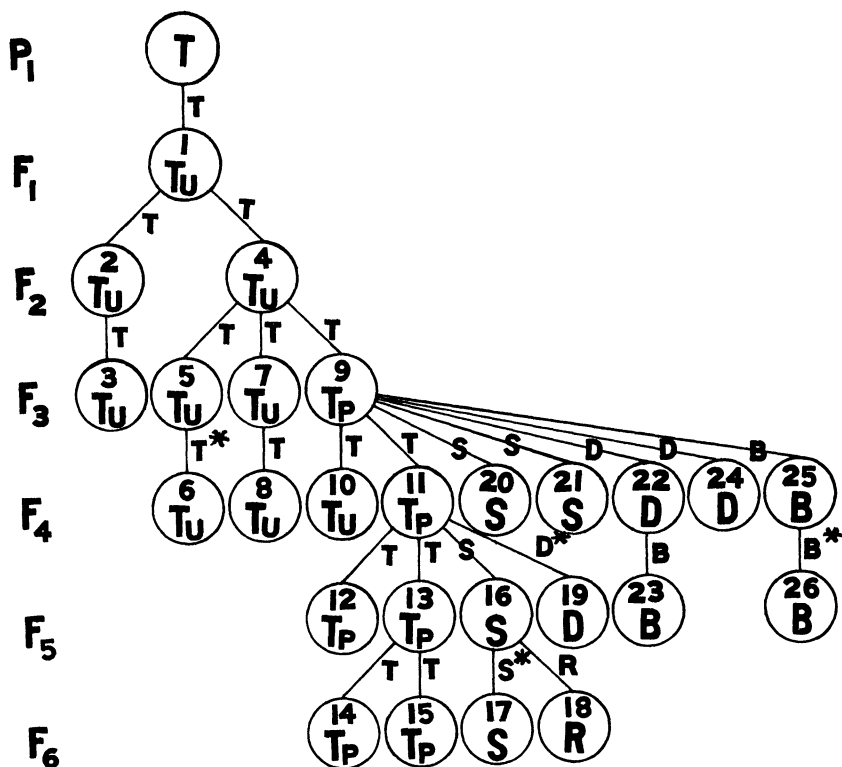


FIG. 1. Pedigree of *Oenothera Reynoldsii* and its mutations. Each numbered progeny is represented by a circle. T = *typica*. S = *semialta*. D = *debilis*. B = *bi-longa*. R = *rigida*. T_u = uniform culture of *typica*. T_p = polymorphic culture containing *typica*. Letters on the lines leading to circles indicate the parentage of the cultures. A star (*) indicates a plant used as a parent for the crosses referred to in Table II.

large as seemed desirable, on which account supplementary cultures were in several cases grown a year or two later from any seeds that had been left over. Such division of progenies between two seasons has provided a very desirable check on the classification of the plants,

TABLE I

Summary of the Pedigree of *Oenothera Reynoldsii* and Its Mutations, Excluding Crosses, up to and Including the Season of 1916

Key No. (See Fig. 1)	Genera- tion from Founda- tion of Pedigree	Genera- tion from Appear- ance of Mutation	Culture No. of Parent	Identity of Parent	Seeds Planted	Germinations		Plants Re- tained to Ma- turity	<i>Typica</i>	<i>Semi- alta</i>	<i>Rigida</i>	<i>Debilis</i>	<i>Bilonga</i>	Other Muta- tions
						No.	%							
1	F ₁	..	91	<i>typica</i>	10	10
2	F ₂	..	01-2	<i>typica</i>	24	23	I
3	F ₃	..	89	<i>typica</i>	110	110
4	F ₂	..	89	<i>typica</i>	105	104	I
5	F ₃	..	89-53	<i>typica</i>	500	419	82%	100	100
6	F ₄	..	89-53-1	<i>typica</i>	750	538	71%	99	98†	I
7	F ₃	..	89-85	<i>typica</i>	870	733	84%	100	100
8	F ₄	..	89-85-40	<i>typica</i>	644	382	59%	100	100
9	F ₃	..	89-3	<i>typica</i>	79	29	31	..	18	I	..
10	F ₄	..	89-3-4	<i>typica</i>	100	100
11	F ₄	..	89-3-13	<i>typica</i>	2,500	111	4.4%	24	5	13	..	5	I	..
12	F ₅	..	89-3-13-14	<i>typica</i>	1,265	64	5.1%	105	20	61	..	21	3	..
13	F ₅	..	89-3-13-15	<i>typica</i>	2,000	92	4.6%	61	12	35	..	11	..	2
14	F ₆	..	89-3-13-15-50	<i>typica</i>	612	14	2.3%	90	22	50	..	13	I	4
15	F ₆	..	89-3-13-15-79	<i>typica</i>	472	20	4.3%	13	9†	2	2
16	F ₅	F ₁	89-3-13-4	<i>semialta</i>	658	584	88.7%	20	3†	13	..	3	..	I
17	F ₆	F ₂	89-3-13-4-16	<i>semialta</i>	962	597	62%	50	..	47	I	2
18	F ₆	F ₁	89-3-13-4-43	<i>rigida</i>	1,891	1,360	72%	100	..	100	200
19	F ₅	F ₁	89-3-13-134	<i>debilis</i>	832	62	7.5%	62
20	F ₄	F ₁	89-3-22	<i>semialta</i>	46	60	2	..
21	F ₄	F ₁	89-3-24	<i>debilis</i>	87	41	5
22	F ₄	F ₁	89-3-21	<i>debilis</i>	85	83	4
23	F ₅	F ₁	89-3-21-85	<i>bilonga</i>	1,084	375*	34.6%*	375	84	I	..
24	F ₄	F ₁	89-3-25	<i>debilis</i>	43	375	..
25	F ₄	F ₁	89-3-53	<i>bilonga</i>	815	196*	24%*	190	42	I	I
26	F ₅	F ₂	89-3-53-22	<i>bilonga</i>	750	194*	25.9%*	150	189	..

* Germination number and percent include only green plants. See p. 127.

† The *typica* individuals divide into two classes, differing only in habit of growth, either annual or biennial. Both come to maturity in one season, but the biennial individuals stay in the rosette stage longer, and therefore produce more robust plants at time of flowering.

but has brought it about that the different lines composing the cultures have not all been carried the same number of generations since the foundation of the pedigree in 1910. The oldest lines have now been carried through six generations by self-pollination.

At the close of the season of 1914 the cultures of *Oe. Reynoldsii* had been maintained for four generations, and the pedigree, as summarized in the former paper,⁶ showed clearly that the individuals of *f. typica*, externally alike, were of two kinds, giving rise, respectively, to uniform and polymorphic progenies. It had been shown, also, that both kinds of *f. typica* occurred in polymorphic progenies. One point, however, was still obscure. The original mass-mutant individual of *f. typica* had been the only plant of its generation self-pollinated for continuing the line, and it was therefore uncertain whether the sister plants of the same culture would have resembled it in giving rise to polymorphic progenies or would have given uniform progenies. In other words, was the original mass-mutant individual of *f. typica* itself of the nature of a rare physiological mutation? In order to answer this question it was necessary to go back to old seeds and retrace two generations. This has been done, with the result that two sister plants of the original mass-mutant individual have given two generations of uniform progeny. (It should be noted that here, as well as in the explanation of the pedigree, Figure 1, a progeny is for convenience termed uniform, as opposed to polymorphic, even if it contains a few mutations, provided the mutability was unaccompanied by unusual seed sterility. The sporadic mutations that have appeared in so-called uniform progenies have in no case been those characteristic of mass mutability. No confusion can possibly arise from this terminology for the reason that Table I gives a detailed analysis of every culture concerned in the experiments.) It is therefore possible to conclude that mutating and non-mutating individuals of *f. typica* may occur together in either uniform or polymorphic progenies. In the former case the mutating individual must itself be regarded as a physiological mutation, or perhaps even as a premutation in the sense of De Vries.⁸

Premutation, according to De Vries, is the process of preparation for mutation. In forms showing ordinary mutability the various mutational types occur in every sufficiently large progeny in every generation, and the process of premutation must therefore be assumed to have taken place far back, and to have brought about a hereditary

⁸ De Vries, *Gruppenweise Artbildung*, pp. 9 and 10, 335, 346.

change by virtue of which all individuals of the line became mutable. The phenomena are not quite comparable in the case of mass-mutant *Oe. Reynoldsii*, and lead one to wonder if the change in the genetic physiology of the original individual of mass-mutant *Oe. Reynoldsii* may not have been a premutation accidentally detected at the actual time of origin. Speculation on such a point, however, will hardly be worth while until the investigations shall have been pushed much further than they have been as yet.

To those who may desire to explain the mutations of *Oe. Reynoldsii* on a Mendelian basis the facts are very refractory. The lines have been grown from guarded seeds since 1911, and have probably been self-pollinated much longer, for the species is one of the smaller-flowered self-pollinating types, producing abundant pollen that is liberated on the receptive stigma a day, or even two days, before the flowers open. If the wild parent plant had been an F_1 hybrid, or a heterozygote of a later generation, the first generation in the garden should have shown segregation, whereas the first polymorphic progeny was obtained two generations later. An explanation based on the multiple factor hypothesis is blocked by the fact that the mutations do not act as Mendelian recessives, but show strict matroclinic inheritance when crossed with the parent type.

SEED STERILITY OF THE MASS-MUTANT INDIVIDUALS

Returning to the problem presented by the two types of individuals of f. *typica*, we see from Table I that there is at least one character by which they may be distinguished. All those plants giving rise to uniform progenies have reasonably good seeds, relatively many of which (58 to 84 percent) readily germinate. Those giving rise to polymorphic progenies, on the contrary, have very poor seeds, few of which (2 to 5 percent) are capable of germinating.

When the seeds for two of the polymorphic progenies (Nos. 11b and 12 in Table I) were counted off it was found that only about 5 percent of the seed-like structures were actually perfect seeds with a good embryo. The remainder were either empty shells, or else contained a small amount of yellowish disintegrated tissue. Many were examined. It is therefore certain that the low germinability of the seeds that yield polymorphic cultures is not to be attributed to delayed germination. If a cytological study now in progress throws any light on the reason for the seed sterility, we may be well on the way

to an understanding of mass mutation. Certainly there is likely to be some causal relationship between such closely associated phenomena.

One must of course take into consideration the possibility that the defective seeds represent zygotes of f. *typica* that failed to develop. Reckoned from the total number of seed-like structures sown, rather than from the number of plants obtained from them, the proportion of mutations in the polymorphic progenies would not be at all unusual. We are not inclined to believe, however, that any such explanation is the right one. Why should the *typica* zygotes in one case develop into uniformly strong and viable embryos, but in another case, environmental conditions remaining the same, fail to produce even mature embryos? Moreover, if there were no essential difference between uniform and polymorphic progenies other than the failure of *typica* zygotes to develop, why should the mutations found in the polymorphic progenies be characteristic of the latter? It may be urged that the evidence is not sufficiently clear that the non-mass-mutant individuals might not throw mutations *semialta*, *debilis* and *bilonga* if grown in sufficiently large cultures. For the present it must suffice to say that they have not done so, although we are keenly aware of the fact that the cultures have not been as large as one would wish for convincing evidence on this point. Very much larger cultures to test this question are planned for next year. It should be remarked that the mutations of *Oe. Reynoldsii* are not sufficiently characteristic in youth to admit of accurate classification, and that consequently every plant of each culture must be carried to maturity if it is to be certainly identified. With most of the other mutable species it is possible to discard many of the typical individuals which make up the bulk of the cultures without giving them garden space, since the young plants are as easily distinguished as the mature ones.

In marked contrast with *Oe. Reynoldsii*, all individuals of f. *typica* in the mass-mutant strain of *Oe. pratincola* seem capable of throwing the mutations characteristic of mass mutation in that species, and such individuals differ among themselves as widely as possible in degree of mutability. Moreover, in *Oe. pratincola* the number of abortive seeds seems to vary in approximately inverse proportion to the number of *typica* individuals obtained from the seeds. This fact might be adduced as an argument for considering the bad seeds as resulting from the abortion of *typica* zygotes. We do not wish to minimize this possibility but prefer for the present the hypothesis that the zygotes which fail to develop represent mutational types of excessively weak constitution.

It appears at present that mass mutation in *Oe. Reynoldsii* differs considerably from the similar process in *Oe. pratincola*, the chief difference being that in the former species the characteristic mutations are produced only by certain individuals of f. *typica* in which there is great seed sterility, whereas in the latter species any individual of f. *typica* belonging to the mass mutant strain may give rise to the characteristic mutations, the mutable individuals differing widely among themselves in mutability and seed sterility. The process is alike in both species in that the characteristic mutations occur only in strains some members of which are excessively mutable (*i. e.*, mass-mutant) and in that the characteristic mutations in both cases show matroclinic inheritance.

Before turning to the evidence in regard to matroclinic inheritance there is a further feature of seed abortion to which attention should be called. The germination data in Table I show clearly that mutations arising from highly infertile mass-mutant f. *typica* are not themselves excessively infertile. The degree of seed abortion is not nearly as great in the mutations as in the parent plant that produced them. Seeds of mass-mutant f. *typica* have given germinations varying from 2.3 to 5.1 percent. In striking contrast to this low viability, seeds of mut. *semialta* have given germinations of 88.7 percent and 62.0 percent; seeds of mut. *rigida*, 72.0 percent. The germinations recorded for mut. *bilonga* are much lower than the true value, because only green plants that survived were counted. This mutation has the curious characteristic of giving rise to progenies consisting of a mixture of green and yellow plants. The latter lack the capacity for chlorophyll production, and die shortly after the cotyledons unfold. The relative numbers of green and yellow plants have not yet been determined. Leaving yellow plants out of consideration, mut. *bilonga* has given progenies numbering 34.6 percent, 24.0 percent, and 25.9 percent of the number of seeds sown—well in excess of the viability of mass-mutant f. *typica*. Complete records have been kept for only one progeny of mut. *debilis*. This form is a weak dwarf, of which the seeds are much less viable than those of the other mutations. Only 7.5 percent of germinations were obtained. It must be remembered, however, that mut. *debilis*, the most sterile of the mutations, gives rise to mut. *bilonga*, a form showing a distinct increase in fertility over its parent. We can not doubt that in the case of the mutations seed sterility is in a large measure inversely proportional to the vegetative vigor of the parent plant.

The same explanation does not hold for the difference between the two kinds of *f. typica*, for vegetatively they are equally vigorous. May not the yellow seedlings which occur in progenies of mut. *bilonga* give a clue to an understanding of the situation? These yellow seedlings constitute a mutational type in which chlorophyll formation can not take place, and therefore a type which can not persist more than a few days after germination. It does not require a very great effort of the imagination to conceive of physiological defects that might originate by mutation and that might operate disadvantageously to the organism possessing them at an even earlier stage in the life cycle than failure to produce chlorophyll. May not the aborting seeds in the polymorphic progenies represent one or more physiologically defective classes of mutations, of which the zygotes are unable to develop into mature embryos? Pending cytological study of the abortive seeds, such a hypothesis seems to us much more plausible than the alternative hypothesis that they are *typica* zygotes, eliminated by some unknown selective process that leaves to develop the intrinsically weaker zygotes of the several mutational types.

MATROCLINIC INHERITANCE IN THE MUTATION CROSSES

In 1915 a complete series of mutation crosses was made, involving *f. typica* and the three well-known mutations. One parent plant of each form served for self-pollination and for crossing with the three other forms. Each cross was made reciprocally. Two of the twelve crosses, mut. *semialta* × mut. *debilis* and mut. *bilonga* × mut. *debilis*, failed, but the remaining ten were in varying degrees successful, and progenies of all were grown in 1916. The reader will find the four parent plants of these crosses indicated by asterisks in figure 1, and may determine by reference to Table I that all gave rise to uniform progenies in the following generation. It will be observed that the phenomenon of mass mutation had not occurred in the direct line of descent of the individual of *f. typica* chosen as a parent. Both the *semialta* and the *bilonga* parents belonged to first generation progenies from primary mutations (*i. e.*, mutations derived directly from *f. typica*, and not from one of the other mutations). The former type arises only as a primary mutation, but the latter is frequently derived as a secondary mutation from mut. *debilis*. The individual of mut. *debilis* used as a parent was an actual primary mutation in a polymorphic progeny, chosen because, at the time the other plants were in

condition for crossing, the uniform first generation culture of mut. *debilis* did not contain a single plant on which enough flowers remained to suffice for all of the crosses. The detailed analysis of the mutation crosses is given in Table II.

In brief, the results of the mutation crosses are as follows:

typica \times semialta \rightarrow typica
 typica \times debilis \rightarrow typica
 typica \times bilonga \rightarrow typica + yellow twin
 semialta \times typica \rightarrow semialta
 semialta \times bilonga \rightarrow semialta
 debilis \times typica \rightarrow debilis
 debilis \times semialta \rightarrow debilis
 debilis \times bilonga \rightarrow debilis + bilonga
 bilonga \times typica \rightarrow bilonga + yellow twin
 bilonga \times semialta \rightarrow bilonga + yellow twin

With one exception the scheme of inheritance is strictly matroclinic. The type of pollen used is immaterial, providing it does not come from mut. *bilonga*. All progenies which did not have mut. *bilonga* as the pollen parent were exactly the same as they would have been if the mother plant had been self-pollinated. The fact has already been mentioned that progenies of self-pollinated mut. *bilonga* consist of a mixture of green and yellow plants. Every cross into which mut. *bilonga* entered as the pistillate parent showed exactly the same mixture of green and yellow plants, of which the former developed as normal mut. *bilonga* and the latter died. It is obvious, however, that the crosses with mut. *bilonga* as pollen parent constitute a real exception to the prevalence of matroclinic inheritance in the mutation crosses.

In the case of mut. *debilis* \times mut. *bilonga* the progeny contained both the maternal and the paternal types, the latter in such large numbers that it was not possible to view them as having arisen *de novo* by mutation from *debilis* eggs. Thus the progeny from the cross contained 18 plants of mut. *bilonga* out of a total of 47 plants. By way of contrast, the progeny of the pistillate parent, mut. *debilis*, self-pollinated, included only two individuals of mut. *bilonga* in a total of 62. Mut. *bilonga* was therefore roughly twelve times as frequent in the cross as in the progeny resulting from self-pollination, —a difference that one must ascribe to the pollen parent. Incidentally, it seems worth while to call attention to the fact, without attempt-

TABLE II
 Summary of the Cultures of Mutation Crosses, Grown in 1916. The Parents of the Crosses are Indicated by Asterisks in Fig. 1,
 and the Composition of Progenies Derived from Them by Self-pollination May be Found in Table I

Progeny No.	Cross	♀ Parent	♂ Parent	Seeds Planted	Germination		Retained to Maturity	Typica	Semi-alba	Debilis	Bilonga	Other Mutations
					No.	%						
27	<i>typica</i> × <i>semialba</i>	89-53-1	89-3-13-4-16	1,021	861	84.3%	200	199	1 (#143)
28	<i>typica</i> × <i>debilis</i>	89-53-1	89-3-13-134	569	458	80.5%	200	200
29	<i>typica</i> × <i>bilonga</i>	89-53-1	89-3-53-22	840	162*	19.3%*	162	162
30	<i>semialba</i> × <i>typica</i>	89-3-13-4-16	89-53-1	593	333	56.2%	200	..	197	3
31	<i>semialba</i> × <i>bilonga</i>	89-3-13-4-16	89-3-53-22	783	221	28.2%	200	..	189	1	10	..
32	<i>debilis</i> × <i>typica</i>	89-3-13-134	89-53-1	184	14	7.6%	14	14
33	<i>debilis</i> × <i>semialba</i>	89-3-13-134	89-3-13-4-16	99	34	34%	34	34
34	<i>debilis</i> × <i>bilonga</i>	89-3-13-134	89-3-53-22	105	52	50.0%	47	29	18	..
35	<i>bilonga</i> × <i>typica</i>	89-3-53-22	89-53-1	153	50	32.7%	50	50	..
36	<i>bilonga</i> × <i>semialba</i>	89-3-53-22	89-3-13-4-16	501	150	30.0%	140	140	..

* Seeds of the cross f. *typica* × mut. *bilonga* gave a mixed progeny of green and yellow plants, apparently in about equal numbers. The yellow plants died off rapidly and were not counted. The germination record therefore takes account of the green plants only.

ing to draw any conclusions from it, that seeds of mut. *debilis* were much less viable than those of the crosses into which this form entered as the pistillate parent.

The progeny obtained from the cross f. *typica* \times mut. *bilonga* showed the influence of the pollen parent in the large number of yellow plants, the same, as far as superficial observation could indicate, as the yellow twin that appears in progenies from self-pollinated mut. *bilonga*. In view of the fact that the crosses *typica* \times *bilonga* and *debilis* \times *bilonga* both resulted in twin types, it is interesting that the third cross, *semialta* \times *bilonga*, gave only plants of the maternal type, aside from a few which appear to be satisfactorily accounted for as derived from mutated gametes.

It will be observed from a scrutiny of Table II that in the foregoing discussion we have tacitly assumed that the sporadic occurrence of types in cultures where they would not necessarily be expected to occur was to be ascribed to mutation. We have made no special comment, for example, on the few individuals of mut. *debilis* that turned up in the progeny of *semialta* \times *typica*, for the reason that mut. *semialta* always seems to produce some mutated gametes that give rise on fertilization to mut. *debilis*. Furthermore, there is every ground for the belief, on evidence furnished by the matroclinic progeny of the cross *debilis* \times *typica*, that the few *debilis*-yielding eggs of *semialta* would give rise to mut. *debilis* quite regardless of the source of the male gametes, provided, of course, that the latter were not derived from mut. *bilonga*, the one form of the series that seems to give rise to more than one type of sperms.

To return to the case of the cross *semialta* \times *bilonga*, we have seen that mut. *semialta* always produces some *debilis*-yielding eggs, and that the cross *debilis* \times *bilonga* yields a progeny containing both parental types. Consequently we should expect that in the cross *semialta* \times *bilonga* some of the mutated eggs would give rise to mut. *debilis* and some to mut. *bilonga*, whereas in a progeny resulting from self-pollination or from one of the other crosses the mutated eggs would be represented by mut. *debilis* alone. The results of the crosses realize this expectation. Although mut. *semialta*, whether self-pollinated or crossed, has always given rise to mut. *debilis*, it has never given mut. *bilonga* except in the case of the cross *semialta* \times *bilonga*.

The results of the whole series of cultures are intelligible on the supposition that *Oe. Reynoldsii* is one of the mutable species to which

De Vries⁹ would apply the term heterogamous. It has frequently been found that crosses of the *Oenotheras* differ strikingly according to the direction in which the cross is made. Often the reciprocal hybrids from the same two parent plants are as unlike as the parents themselves. De Vries has attributed such results to a difference in the hereditary qualities of the male and female gametes, and has suggested the term heterogamy for the condition of species in which such a differentiation of gametes is found. There is much unpublished evidence at hand which tends to show that heterogamy may exist in some species without a sharp restriction of either type of gamete to the eggs or sperms and on this account we shall use the term "heterogamy" with no implication that the non-equivalent gametes may not exist on both the male and female sides. The conception of heterogamy so modified as to apply to results that have been obtained in our experiments has been published¹⁰ in advance of the data which suggested the modification.

Let us assume (1) that a heterogamous species such as *Oe. Reynoldsii* normally produces two types of non-equivalent gametes, which may be designated as α and β respectively; (2) that the α gametes carry most of the characters by which specific differentiation is effected; (3) that mutation occurs through the modification of α gametes, which thus become α' , α'' , α''' , etc. Applying this conception to the particular case in hand, let us think of f. *typica* as the zygote $\alpha\beta$, mut. *semialta* as $\alpha'\beta$, mut. *debilis* as $\alpha''\beta$, and mut. *bilonga* as $\alpha'''\beta$. The conditions imposed by the results of the various crosses are satisfied if

f. *typica* $\rightarrow \alpha$ eggs + β sperms,
 mut. *semialta* $\rightarrow \alpha'$ eggs + β sperms,
 mut. *debilis* $\rightarrow \alpha''$ and β eggs + β sperms,
 mut. *bilonga* $\rightarrow \alpha'''$ and β eggs + α''' and β sperms.

Since the various forms are determined by the α gamete, all mutation crosses must of necessity show matroclinic inheritance, except those involving mut. *bilonga*, for this one form is the only member of the series that produces any male α gametes. In order to be functional, male α gametes must fuse with female β gametes, which are produced only by mut. *debilis*. Therefore the cross *debilis* \times *bilonga* is the only one that yields both the maternal and paternal types. It will

⁹ De Vries, Gruppenweise Artbildung, pp. 30-32.

¹⁰ Bartlett, H. H., The Status of the Mutation Theory, with Especial Reference to *Oenothera*, Amer. Nat., 50: 513-529. 1916.

be remembered that mut. *debilis*, when self-pollinated, was marked by great seed sterility. This sterility was much reduced when pollen from one of the other forms was used, and the effect of foreign pollen was greatest of all when that of mut. *bilonga* was used. Doubtless several factors are concerned with the increase of fertility on crossing, but it seems not unwarranted to call attention to the fact that if our hypothesis were true such an increase would be expected, because good embryos would result from the fertilization of female β gametes by male α gametes. All the *bilonga* individuals in the mixed progeny from *debilis* \times *bilonga* would be represented in a self-pollinated progeny by aborted seeds.

On the whole, the facts point to the truth of the hypothesis of non-equivalent gametes. The facts to be explained are sufficiently orderly to demand more than a superficial criticism at the hands of those who see in the mutation phenomena merely evidence of Mendelian segregation. It seems to the writers that the work with *Oe. Reynoldsii* affords very convincing evidence of De Vriesian mutation.

QUANTITATIVE EVIDENCE OF MATROCLINIC INHERITANCE

Although no one who has had an opportunity to examine the mutation crosses has doubted the fact of matroclinic inheritance, it was of course essential to obtain quantitative data that would convince one of the accuracy of our observations. Leaves and capsules from self-pollinated and crossed progenies were therefore measured, both in order to establish the fact that the several forms differed widely from one another and to show that the mutation crosses resembled the pistillate parent. In most cases a large enough number of plants was at hand to give satisfactory data.

Mature stem leaves were measured from plants of all the pure strains and mutation crosses, five leaves being taken at the same part of the main stem from each plant as it came in the row, without selection. The leaf lengths are summarized in Table III, the widths in Table IV. The two tables are based upon the same material, but individual leaves were frequently imperfect, so that one or the other measurement could not be made. On this account the number of measurements does not always tally in the two tables. It is very clear that the modes of the variation curves lie very close together in the cases of all progenies having the same pistillate parent. There are some discrepancies, to be sure, the most notable being the failure of a closer

agreement between *semialta* \times *semialta* and *semialta* \times *typica*, and, conversely, the unexpected closeness of the modes for *semialta* \times *typica* and *typica* \times *semialta*. It is believed, however, that the departures from the expected are all explained by environmental non-uniformity. The garden itself was relatively uniform, but the cultures were set out at different times, so that some of the plants were subjected to hot dry weather much sooner than others. The most rapid growth took place early in the season, with the result that the last plants to be transplanted were markedly the weakest. Without exception, the greater deviations from the measurements which would have been expected in matroclinic inheritance were correlated with the greater intervals between time of transplanting, and, conversely, the best agreements with expectation were found in the cases of cultures set out on the same day.

A valuable evidence of shifting of the mode attributable to difference in date of transplanting was quite accidentally obtained in the case of the cross *bilonga* \times *semialta*. The culture had been partly transplanted at the close of the day, and the next morning the remainder was overlooked. It was set out a couple of weeks later. Leaves from the two lots were collected separately, and the data are given separately in Tables III and IV.

Considering the unfavorable experimental conditions, the measurements provide as good a demonstration of matroclinic inheritance as could reasonably be demanded. Moreover, it would be unfair not to emphasize the fact that in the one or two cases where the measurements might appear ambiguous, the plants were in general aspect true to the expected type. Thus the cross *semialta* \times *typica* had the whole appearance of self-pollinated *semialta*, from which it differed only in being much more robust. Although we are of the opinion that environmental non-uniformity explains the difference, we shall carry out more carefully controlled experiments to see if cross-pollination produces any effect similar to the vigor of heterozygosis. Such an effect might conceivably be measurable if environmental non-uniformity were eliminated, but if it exists it is obviously not large enough to obscure the underlying phenomenon of matroclinic inheritance.

The data for capsule length in the mutation crosses are incomplete because of the fact that some of the cultures of 1916 were just coming into flower at the time of the first heavy frost and were destroyed. Before examining the data for the crosses, the reader should glance at

Table V, which shows the range of variation in capsule length in each of the four pure forms. The measurements were made in Maryland in 1915. In the cases of f. *typica* and mut. *semialta* the ten lowest normal capsules of the main inflorescence of each plant were used. The capsules of mut. *debilis* were taken from the *debilis* plants of a polymorphic progeny. They were collected at random, because very few inflorescences bore as many as ten good fruits. Capsules of mut. *bilonga* were measured from two different cultures, in order to demonstrate the essential identity of this mutation, whether obtained directly from f. *typica*, or as a secondary mutation from mut. *debilis*. The measurements prove the anticipated identity, or, if anything, give a false impression that the secondary mutation is stronger than the primary. This impression is due to the fact that at the time the measurements were made the secondarily derived mut. *bilonga* had been so short a time in flower that ten full-grown capsules could not be obtained from each inflorescence. Six fruits were therefore taken from each, and the greater average length which they show in comparison with primarily derived mut. *bilonga* is due to their lower position in the inflorescence. The progeny of primary mut. *bilonga* was arbitrarily divided into a class of weak plants and a class of strong. The capsules of these two classes, ten from each plant, were separately measured, and the results are recorded in Table V for each class separately and for the two classes combined. The discrepancy between the two classes was much less than was expected, and indicated clearly that the capsules respond less to environmental conditions than vegetative parts of the plant. Measurements of any other part would have shown a much more marked difference between the arbitrarily selected strong and weak plants.

On account of its relative independence of environmental factors the capsule length affords us a more conservative criterion of matroclinic inheritance than the leaf measurements which have already been considered. In this respect it seems to be similar to the character of flower size, which East¹¹ has found especially useful in his studies of inheritance of quantitative characters in *Nicotiana*. In *Nicotiana sylvestris* he found that adverse environmental conditions, which brought about a shortening of the leaf amounting to three fourths of its normal length, did not appreciably change the size of the flowers.

¹¹ East, E. M., Inheritance of Flower Size in Crosses between Species of *Nicotiana*, Bot. Gaz. 55: 177-188. 1913.

TABLE V
Length of Capsule in Oe. Reynoldsii and its Mutations

Length in Mm.	<i>Typica</i> Progeny No. 7	<i>Semialta</i> No. 16	<i>Debilis</i> No. 11	<i>Bilonga</i> from <i>Debilis</i> No. 23	<i>Bilonga</i> from <i>Typica</i> : Weak No. 25	<i>Bilonga</i> from <i>Typica</i> : Strong No. 25	<i>Bilonga</i> from <i>Typica</i> : Sum No. 25
14-15	7
16-17	38
18-19	86
20-21	6	105
22-23	43	104
24-25	97	84
26-27	122	51
28-29	94	31
30-31	4	57	12
32-33	21	21	6
34-35	65	13	3
36-37	165	2
38-39	260	1
40-41	223	1
42-43	82	1
44-45	10	0	3	3
46-47	4	14	5	19
48-49	8	24	18	42
50-51	49	41	54	95
52-53	87	72	87	159
54-55	122	64	91	155
56-57	130	53	89	142
58-59	141	21	60	81
60-61	110	11	49	60
62-63	71	7	31	38
64-65	36	6	21	27
66-67	18	3	5	8
68-69	5	1	6	7
70-71	3	3	3
72-73	1	0	0
74-75	1	1

Goodspeed and Clausen¹² have questioned East's conclusions, and have demonstrated beyond question that the flower size does respond to environmental changes, but their results really strengthen the contention, that, as compared with changes in other parts, the flowers are relatively little affected. Our own conclusion in regard to the fruits of *Oenothera* is that they respond in size to varying environment, but that the response is relatively much less than the response in height of plant or size of leaves, and that the character of capsule length is particularly significant as a criterion of matroclinic inheritance.

¹² Goodspeed, T. H., and Clausen, R. E., Factors Influencing Flower Size in *Nicotiana*, with Special Reference to Questions of Inheritance, Amer. Journ. Bot. 2: 332-374. 1915.

TABLE VI

Capsule Lengths of Oe. Reynoldsii f. typica and mut. semialta, and of Some of their Mutation Crosses

The capsules measured were the lowest five capsules from each of two secondary inflorescences from each plant; the progeny numbers refer to Tables I and II.

Length in Mm.	F. <i>Typica</i>				Mut. <i>Semialta</i>		
	× <i>Typica</i> Progeny No. 8	× <i>Semialta</i> No. 27	× <i>Debilis</i> No. 28	× <i>Bilonga</i> No. 29	× <i>Semialta</i> No. 27	× <i>Typica</i> No. 30	× <i>Bilonga</i> No. 31
16-17	3	2	3
18-19	5	17	14
20-21	30	59	27
22-23	56	99	37
24-25	I	3	I	114	147	44
26-27	3	5	12	97	124	36
28-29	18	23	11	86	84	29
30-31	44	4	46	28	45	31	10
32-33	79	16	73	54	12	3	7
34-35	100	39	87	71	2	4	2
36-37	86	61	99	90	I
38-39	48	92	82	110
40-41	13	77	37	43
42-43	6	50	14	26
44-45	2	41	6	14
46-47	17	5	4
48-49	9	I
50-51	4

Table V brings out clearly the fact that the forms of *Oe. Reynoldsii* differ distinctly from one another in capsule length. It is not, however, strictly comparable with Table VI. In the first place, the garden of 1915 was in Maryland, where climatic, cultural and soil conditions were unlike those in Michigan. In the second place, the early frost in 1916 overtook the plants before the inflorescence of the main stem was sufficiently mature to provide full-grown capsules. Since the inflorescences of the long basal branches had begun to flower several days earlier, their lower capsules were full-grown. The five lower capsules from two branches of each plant were measured. Thus each plant provided ten capsules, but they were from two lateral branches rather than from the main stem. The capsules of the terminal inflorescence of the main stem are usually slightly larger than any others, and on this account the modes in Table V ought to be higher than in Table VI, as indeed they are. Perhaps the difference is not as great as it would have been if the capsules of the lateral branches had not



FIG. 2. Inflorescences showing mature fruits of the mutation crosses *semialta* \times *bilonga* (left) and its reciprocal, *bilonga* \times *semialta* (right). Each cross is matroclonic, and therefore the lengths of the fruits are in the ratio 1:2.

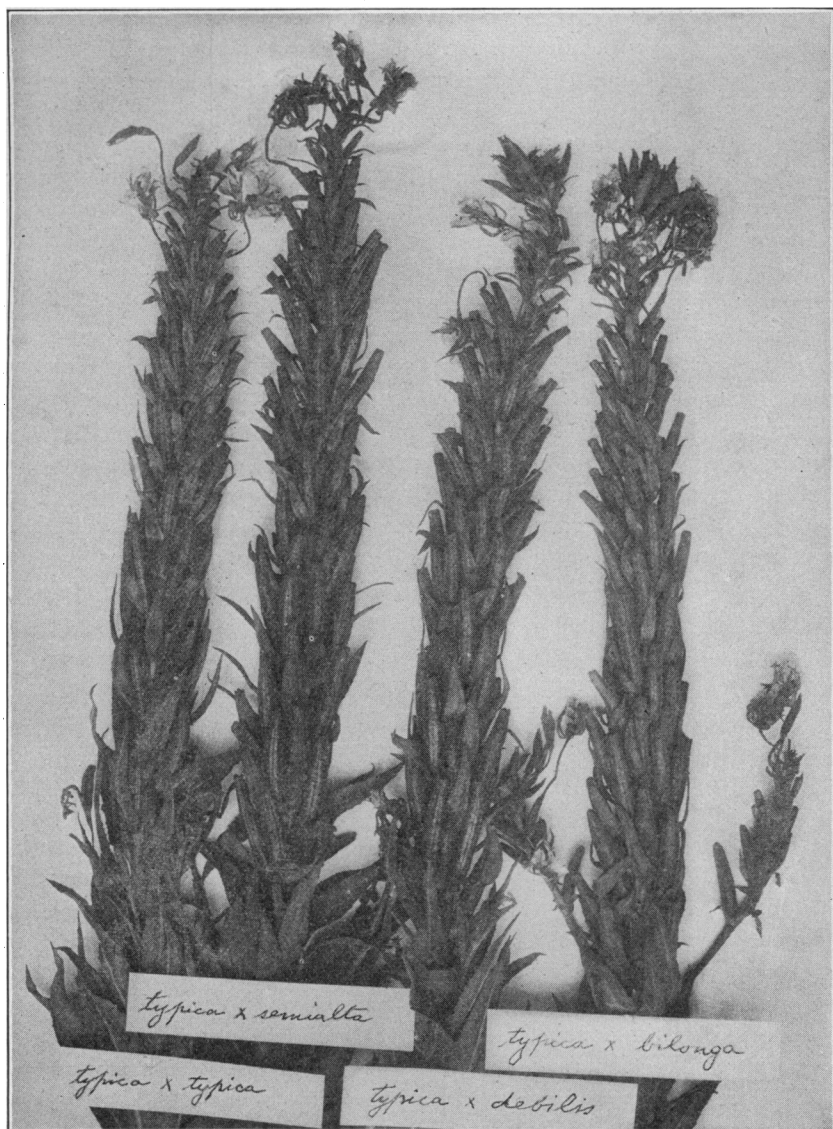


FIG. 3. The *typica* series of mutation crosses. The inflorescences are alike, resembling in each case the pistillate parent, *f. typica*. From left to right, *f. typica* (self-pollinated), *typica* \times *semialta*, *typica* \times *debilis*, and *typica* \times *bilonga*.

had a lower average position in the inflorescence, for the lower capsules, if normally developed, are usually longer than those higher up.

Notwithstanding the difference in material, it is instructive to observe the close agreement between the two sets of measurements. The difference between the modal lengths for f. *typica* and mut. *semialta* in 1915 (Maryland) was just 12 mm., the same as the average difference between the *typica* series of cultures and the *semialta* series in 1916 (Michigan).

The capsule measurements give thoroughly satisfactory evidence of matroclinic inheritance. The data for the *semialta* series of cultures are particularly convincing. The modal lengths are the same for pure *semialta*, *semialta* \times *typica*, and *semialta* \times *bilonga*, being 25 mm. in each case. The ratio of the capsule lengths of the three pollen parents is 2:3:4, but the self-pollinated mut. *semialta* is just like the two crosses. Turning to the slightly less consistent data for the *typica* series, the evidence is hardly less satisfactory. In the cross *typica* \times *debilis* the capsule length is actually slightly higher than in pure f. *typica*, in spite of the shorter capsule of the pollen parent. In the cross *typica* \times *bilonga* the length is slightly greater than in self-pollinated f. *typica*, but that the difference is not significant is certain from the fact that the cross *typica* \times *semialta* has capsules just as long. In the one case the pollen parent has a longer, in the other case a shorter, capsule than the pistillate parent, but the crosses are identical. The results of the capsule measurements, taken all in all, prove that matroclinic inheritance is the rule in the mutation crosses under consideration, and leads us to suspect that there may be such a thing as increased vigor due to cross-pollination, independently of factorial recombinations such as those that occur in Mendelian inheritance.

In the case of the very interesting dimorphic culture resulting from the cross *debilis* \times *bilonga*, it is especially unfortunate that capsule measurements were not obtained, because the two forms differ so much from one another. That measurements would have fully substantiated the conclusions already drawn in regard to this cross is certain from the few precocious plants that matured before frost.

Figure 2 shows typical plants of the cross *semialta* \times *bilonga* and its reciprocal. Of the latter there were too few plants that matured to make a series of measurements worth while. The fact of matroclinic inheritance is obvious from the photograph, however, for in *semialta* \times *bilonga* the capsules are only half as long as in *bilonga*



FIG. 4. The *semialta* series of mutation crosses. From left to right, mut. *semialta* (self-pollinated), *semialta* \times *typica*, and *semialta* \times *bilonga*.

\times *semialta*. Figures 3 and 4 illustrate the *typica* series and the *semialta* series of crosses, respectively.

CONCLUSIONS

1. Mass mutation in *Oenothera Reynoldsii* consists in the production of inordinate numbers of mutations, belonging to several characteristic types, by certain mass-mutant individuals, which may be looked upon as having undergone a premutative modification.

2. Aside from their mutability, these mass-mutant individuals resemble normal f. *typica*. The production by them of a large number of abortive seeds may itself be looked upon as one of the manifestations of mutability.

3. The characteristic mutations form a series, each member of which may give rise to the succeeding member. Thus:

mut. *semialta* \rightarrow mut. *debilis*,
mut. *debilis* \rightarrow mut. *bilonga*.

4. Mut. *semialta* and mut. *debilis* appear to represent successive reduction stages in the mutation series. Mut. *bilonga*, on the contrary, marks an advance over the other members of the mutation series and also over f. *typica*.

5. With the exception of crosses involving mut. *bilonga*, the mutation crosses are matroclinic.

6. The cross *debilis* \times *bilonga* gives a mixture of the two parental types.

7. The facts of inheritance are best explained by the hypothesis that two types of non-equivalent gametes, designated as α and β gametes, are normally produced.

8. The α gametes are usually eggs, and the β gametes sperms, but mut. *bilonga* produces both α and β sperms.

9. Mutation in *Oenothera Reynoldsii* consists in the modification in α gametes of factors that have no counterpart in the β gametes.

10. Since the sperms of f. *typica* are β gametes, mutations appear whenever a mutated α gamete is fertilized. They do not appear as a result of segregation.